

MINUTES

MEETING OF THE S-9 TECHNICAL ADVISORY COMMITTEE

FOR

PLANT GENETIC RESOURCES CONSERVATION AND UTILIZATION
S-9 MULTISTATE RESEARCH PROJECT

A Cooperative Project Among:

THE STATE AGRICULTURAL EXPERIMENT STATIONS
OF THE SOUTHERN REGION

And

U. S. DEPARTMENT OF AGRICULTURE AGENCIES:

AGRICULTURAL RESEARCH SERVICE

COOPERATIVE STATE RESEARCH, EDUCATION, AND
EXTENSION SERVICE

NATURAL RESOURCES CONSERVATION SERVICE

26-28 JUNE 2001

NATIONAL SEED STORAGE LABORATORY
COLORADO STATE UNIVERISTY
FT COLLINS, COLORADO

SUBMITTED BY

KEN QUESENBERRY, CHAIRMAN

Adopted Agenda:

Tuesday afternoon – June 26, 2001

Call to order, 1:00 pm, and opening remarks. Dr. Ken Quesenberry, Chairman

Introduction of attendees

Welcome and remarks – Dr. G. F. Arkin, Assistant Dean, University of Georgia, and
S-9 Administrative Advisor

Approval of minutes – 2000 meeting

Additions to the Agenda

Appointment of committees

Nominations

Time & place of next meeting

Status of PGRCU and future plans – Gary Pederson, Research Leader

Reports by Curators

Bob Jarret

Graves Gillaspie

Roy Pittman

Brad Morris

John Erpelding

Ornamental Plant Germplasm Center – Susan Stieve (Curator) and David Tay
(Director)

Reports from S-9 members on state germplasm research and GRIN data

Wednesday morning, 8:00 a.m., June 27, 2001

Continue reports/discussion among S-9 members regarding germplasm activities

Committee reports

Nominations

Time and place of next meeting

Adjourn

Attendees:**TAC Members:**

Ken Quesenberry, Chair	FL - University of Florida
Gerald Arkin, Administrative Adv.	GA – University of Georgia
David Coffey	TN - University of Tennessee
Don LaBonte	LA - Louisiana State University
Bill Rhodes	SC - Clemson University
Charles Taliaferro	OK - Oklahoma State University
Thomas Zimmerman	VI – University of the Virgin Islands

Griffin PGRUCU Staff:

Gary Pederson, Research Leader
John Erpelding, Sorghum Curator
Graves Gillaspie, Vigna Curator
Brad Morris, Annual Clovers & Special Purpose Legumes Curator
Roy Pittman, Peanut Curator
Merrelyn Spinks, GRIN Coordinator & Computer Support

Other Attendees:

Ricardo Goenaga, USDA-ARS, National Germplasm Repository, Tropical Agricultural
Research Station, Mayaguez, PR
Peter Bretting, USDA-ARS National Program Leader for Germplasm

Recorded Minutes:

The Regional S-9 Technical Advisory Committee was called to order at 8:00 a.m. on Tuesday 26 June 2001 by chairman Ken Quesenberry. The meeting convened in the National Seed Storage Laboratory conference room 251 on the campus of Colorado State University, Ft. Collins, CO.

Dr. Gerald Arkin welcomed the TAC attendees, provided an overview of the S-9 project, and commented on the status of the program. Dr. Arkin reminded the group that the S-9 project is the oldest of its kind, having existed for 52 years. The project must be renewed every 5 years by Southern Regional Agricultural Experiment Station Directors. Dr. Arkin expressed pleasure that positive actions had occurred over the past year addressing major concerns with the Griffin PGRCU program. The first of these was the hiring of Dr. Gary Pederson as Research Leader for the program. A second significant action was an increase in USDA funding for the program. Dr. Arkin noted that the increase was not nearly enough to satisfy the needs of the program, but was a welcome change following a lengthy period of no increases. Dr. Arkin also indicated that good progress was being made addressing some of the difficult questions concerning the handling of germplasm collections, particularly the very large sorghum collection.

Dr. Quesenberry presented the minutes of the 2000 meeting and asked for corrections and additions. The minutes were approved as presented.

Dr. Gary Pederson provided an overview of his vision for the PGRCU and informed TAC members of some of the issues that he had addressed since assuming the RL position. He stated his belief that the fundamental responsibility of the PGRCU is to preserve genetic diversity in the collections. Incumbent in this is the need to develop the knowledge and financial resources to effectively and efficiently achieve this basic goal. Dr. Pederson indicated that he had spent substantial time dealing with problems associated with the sorghum collection and that progress was being made. He indicated that he would fill the grass curator position, left vacant by the retirement of Gil Lovell, at the earliest possible time. He said that the position would probably be filled with a category 3 scientist, but a final decision had not been made. He expressed a concern that the PGRCU receives back very little information on germplasm supplied to users. Such information could greatly enhance the GRIN database for the collections. (Appendix 1)

Updates on curatorial activities were given by Graves Gillaspie (Vigna - Appendix 2), Brad Morris (Clovers & Special Purpose Legumes and Grasses – Appendix 3), Roy Pittman (Peanuts – Appendix 4), and John Erpelding (Sorghum).

Dr. David Tay and Ms. Susan Stieve, Director and Curator, respectively, Floral and Ornamental Plant Germplasm Center, Columbus, OH gave an overview of the newly established center. The center is available to accept floral and ornamental germplasm accessions from existing collections and will augment germplasm of these plant groups through exploration and collection.

There was discussion regarding how to get more evaluation information back from germplasm users. Ideas included trying to identify the intended use of germplasm by those requesting it and then including a request with the shipment for sharing of data in those cases where predicted use would generate useful data. The following motion was made by Quesenberry, seconded by Rhodes, and passed by the committee: 'The S-9 TAC recommends that the germplasm request page on the GRIN web site be modified to include specific categories of intended use'. Such categories would be developed by PGRCU staff and TAC members.

The addition of liaison representatives from Industry and select public agencies (such as the USDA-NRCS) to the TAC was briefly discussed. Taliaferro, incoming Chair of the TAC, will contact the Southern Seed Association and NRCS to determine their interest in having a representative on the committee. This information will be channeled to Dr. Arkin, who will make formal contact with the organizations and arrange for their representation on the TAC.

Arnie Tschanz, APHIS, National Plant Germplasm Quarantine Lab, announced in the morning joint meeting that a review of quarantine regulations would soon be launched. Taliaferro noted that current quarantine policies/regulations place severe restrictions on the importation of clonally propagated plants. Discussion centered on the need for a thorough review of the plant quarantine regulations, particularly an assessment of currently restricted plants and justification for retention of specific plant groups in the restricted category. The committee asked that a request be conveyed to Mr. Tschanz to keep RTAC's advised of developments during the review and that the RTAC's be provided opportunity to examine and comment on drafts of new regulations. This request was voiced in the final joint meeting of the group on Thursday, June 28, 2001.

Quesenberry noted that the S-9 TAC is asked to review germplasm collection proposals. This has usually been done by one representative committee member, but there apparently are not any TAC policy/guidelines addressing the selection of that representative committee member. A motion by Coffey, with second by Rhodes, to designate the outgoing TAC Chair as the representative to review the proposals passed unanimously.

The nominations committee recommended Tom Zimmerman as incoming Secretary and Charles Taliaferro as incoming Chair. The recommendation was unanimously approved.

It was decided that the TAC would meet in Griffin, GA next year. Tentative dates for the meeting are August 5 through 9, 2002.

Dr. Arkin indicated that he would review the membership of the TAC and determine if any new appointments are warranted.

The meeting adjourned at 9:15 a.m.

Appendix 1

DR. GARY PEDERSON

PLANT GENETIC RESOURCES:
CURRENT STATUS & FUTURE RESOURCES

Plant Genetic Resources: Current Status & Future Plans

Gary A. Pederson
USDA, ARS, Plant Genetic Resources
Conservation Unit
Griffin, GA

Outline

- Background
- NPGS mission
- PGRCU mission and purpose
 - General thoughts on different aspects of mission
- Current status of each crop
- Plans for future
 - Staffing
 - Direction

Background

- ARS research geneticist at Miss State for 18 years
- White clover breeding and genetics
- Genetic resources activities
 - Clover CGC (Chair)
 - Collected 49 clover species in mountains of western Bulgaria with Ken Quesenberry in 1993
 - Participated in three CGC Chairs meetings
 - Other legumes crop registration committee (Chair)
 - Eight research proposals funded by Clover CGC
 - Published on cleaning up duplicates in NPGS

What is the mission of NPGS?

- *Primary* mission = preserve indefinitely the genetic diversity of all plant species of interest and their wild relatives.
- Focus of NPGS must be long term, on plant germplasm preservation for future generations, rather than short-term.

Goal should be an “Ideal Collection”

- Ideal collection = complete range of genetic diversity for every plant species of interest and their wild relatives.
- Little duplication or redundancy.
- All accessions would be available, maintained in high quality, and completely characterized.

Why an ideal collection?

- Users, who are our customers, expect it.
- Users expect:
 - complete range of genetic variability
 - little duplication
 - complete availability
 - good germination
 - complete characterization

Goals and financial reality

- Obviously, an ideal collection could not be achieved without significantly greater financial resources.
- Goals should not always be limited by financial reality. That is why they are called “goals”.
- Definition of a goal: *An end that one strives to attain.*

What is the mission of PGRCU?

- A Unit working on Plant Genetic Resources Conservation exists to conserve plant genetic resources.
- Mission statement: “acquire, characterize, maintain, evaluate, document, and distribute genetic resources”.
- This is what the users of the germplasm maintained at this location expect from this Unit.

Thoughts on PGRCU Mission

- Acquire
- Characterize
- Maintain
- Evaluate
- Document
- Distribute

Acquire

- Acquire to increase genetic diversity; not merely to increase collection size.
 - No longer can afford to accumulate everything.
- Avoid acquiring new accessions that duplicate accessions already in the collection.

Characterize

- Users expect characterization of accessions to assist them in making selections.
 - Classical passport information
 - Traits of interest
 - Molecular methods

Maintain

- Is every current accession valuable?
 - Too often once an accession has a PI number, it becomes untouchable.
- Duplicate and redundant accessions add nothing to the genetic diversity of the collection.

Maintain

- Duplicate accessions are a waste of resources for both NPGS and users.
 - NPGS wastes time and resources maintaining, regenerating, backing up, characterizing, and distributing seed of identical accessions.
 - Users waste time and resources evaluating identical material.

Maintain

- Increase the availability of accessions.
 - Base regeneration priority on relative potential for genetic diversity improvement.
- Core collections
 - Core accessions must be readily available and well characterized.

Evaluate

- Prioritize research based on:
 - National priorities
 - Public interest
 - Uniqueness of the evaluation
 - Future usefulness of the germplasm
 - Availability of germplasm
- Cooperate, cooperate, cooperate

Document

- Germplasm records should be complete.
 - Poor record keeping reduces usefulness of accessions.
- GRIN
 - GRIN should contain all documentation previously published in the PI books.
- Data obtained by cooperators must be added to GRIN.

Distribute

- Distribution is the only direct contact this Unit has with many users.
 - Timely, accurate distribution is expected by all users.
 - Users often wait until the last minute to request seed.

PGRCU Collection - June 2001

- Total Accessions

- 81,660

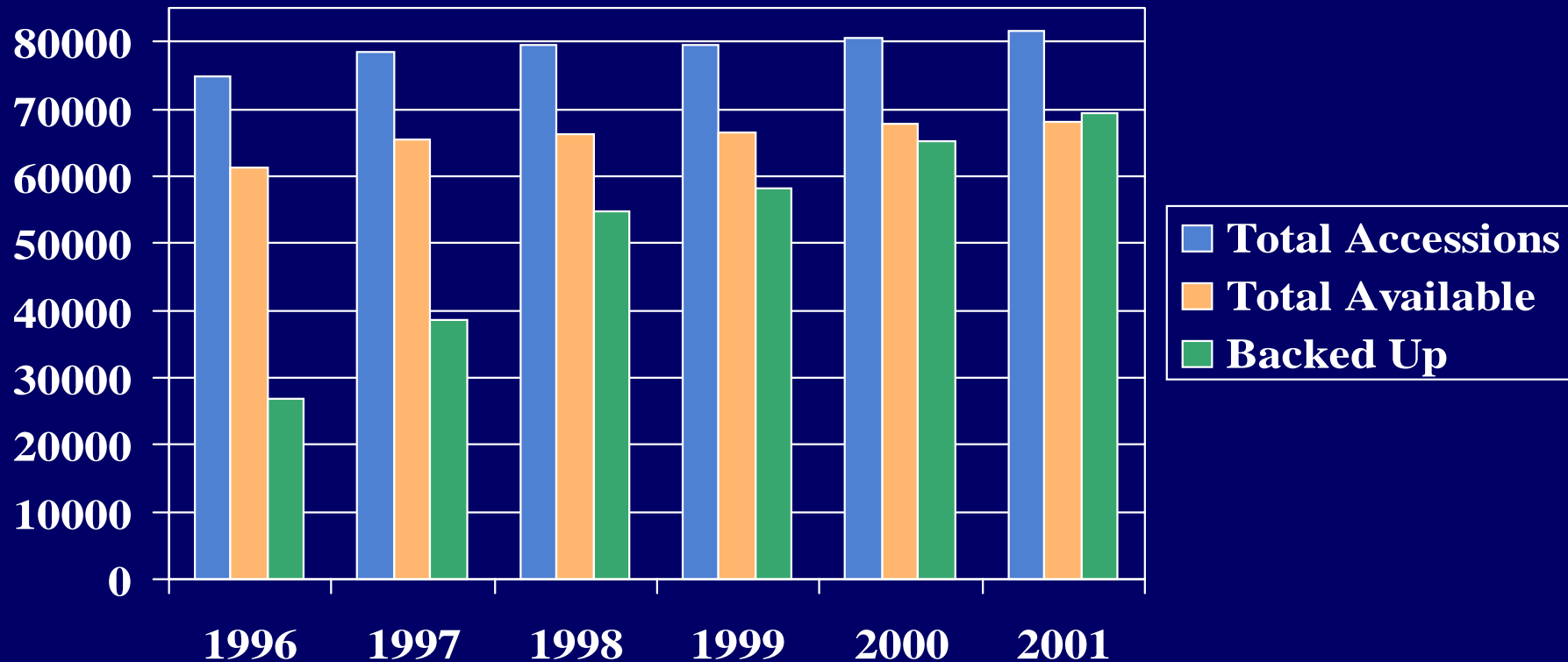
- Total Available

- 68,163

- Backed Up

- 69,483

PGRCU Collection 1996 - 2001



Vigna

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2000
Graves Gillaspie	Cowpea	8,030	5,246	5,885	2,157
	Mung bean	4,194	3,836	4,093	122
	Other Vigna spp.	598	267	296	163

Vegetable Crops & Sweetpotato

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2000
Bob Jarret	Cucurbits	2,017	894	1,301	384
	Eggplant	963	892	923	339
	Okra	3,003	1,538	1,913	178
	Peppers	3,903	3,712	3,802	3,069
	Sweetpotato	715	685	83	208
	Other Ipomoea spp.	422	132	140	42
	Watermelon	1,633	1,539	1605	1,022

Legumes, Grasses, & Other

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2000
Brad Morris	Bamboo	97	97	50	91
	Castor bean	372	279	356	106
	Grasses	6,803	5,829	5,935	1,432
	Kenaf & Roselle	345	276	311	79
	Legumes	3,500	2,653	2,732	720
	Miscellaneous	276	213	236	58
	Pearl millet	1,081	1,048	1,064	69
	Sesame	1,204	1,195	1,204	1,684

Clover & Sorghum

CLOVER CURATOR/ SORGHUM COORDINATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2000
Gary Pederson	Annual Clover	2,088	1,422	1,491	873
	Sorghum	30,705	28,438	27,754	26,729

Peanuts

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2000
Roy Pittman	Cultivated Peanuts	9,027	7,391	8,122	9,609
	Wild Peanuts	684	563	188	123

Requested for regeneration FY2001

Crop	# accessions	Crop	# accessions
Cowpea	394	Grasses	106
Mung bean	12	Kenaf	9
Cucurbit	42	Legumes	214
Okra	97	Misc. crops	32
Peppers	80	Sesame	2
Watermelon	170	Cult peanut	721
Castor bean	1	Wild peanut	23

Domestic Distributions in CY2000

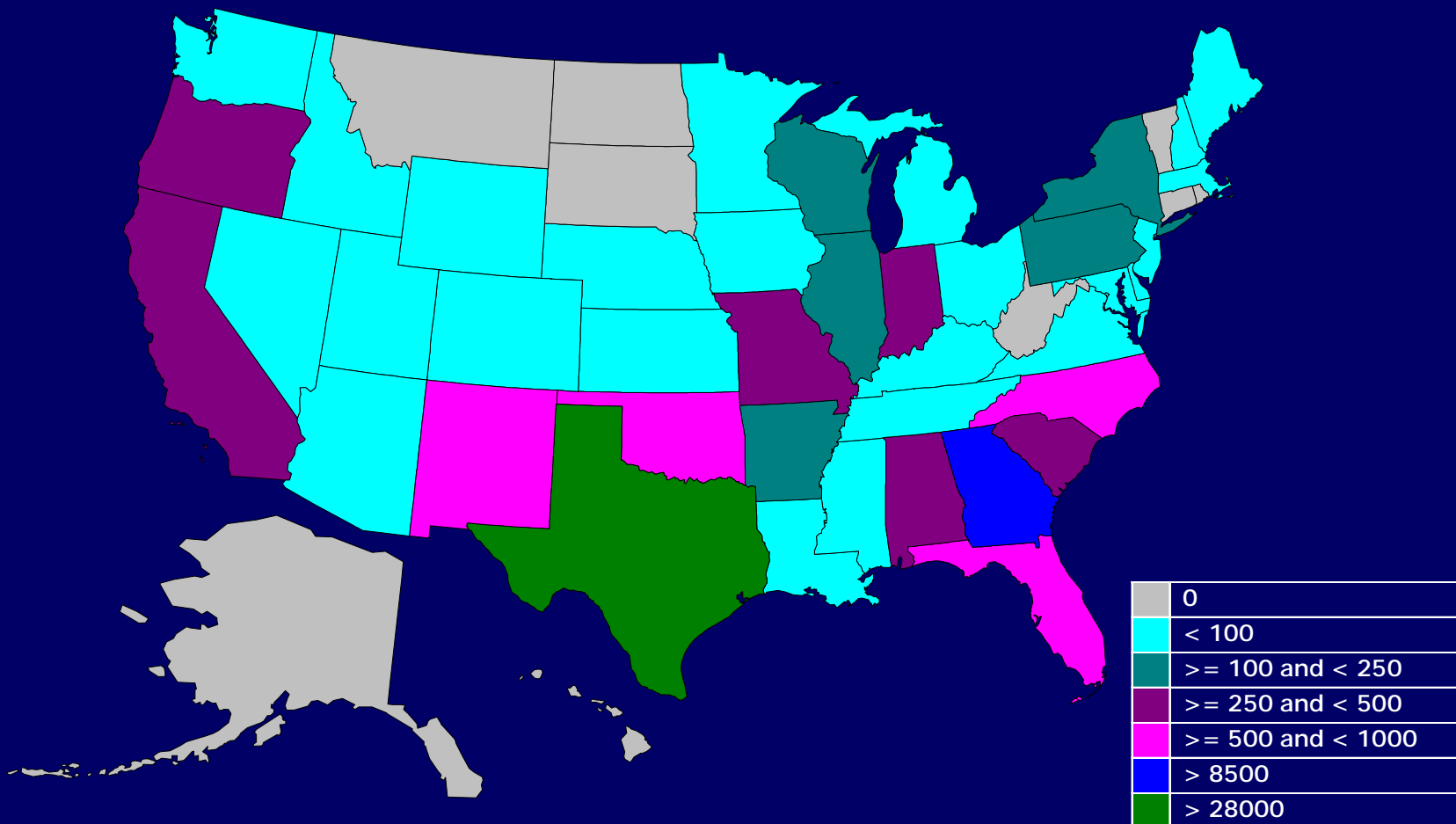
■ Items shipped

– 43,927

■ Orders

– 427

Domestic Distributions in CY2000



Distributions to S-9 Region in CY2000

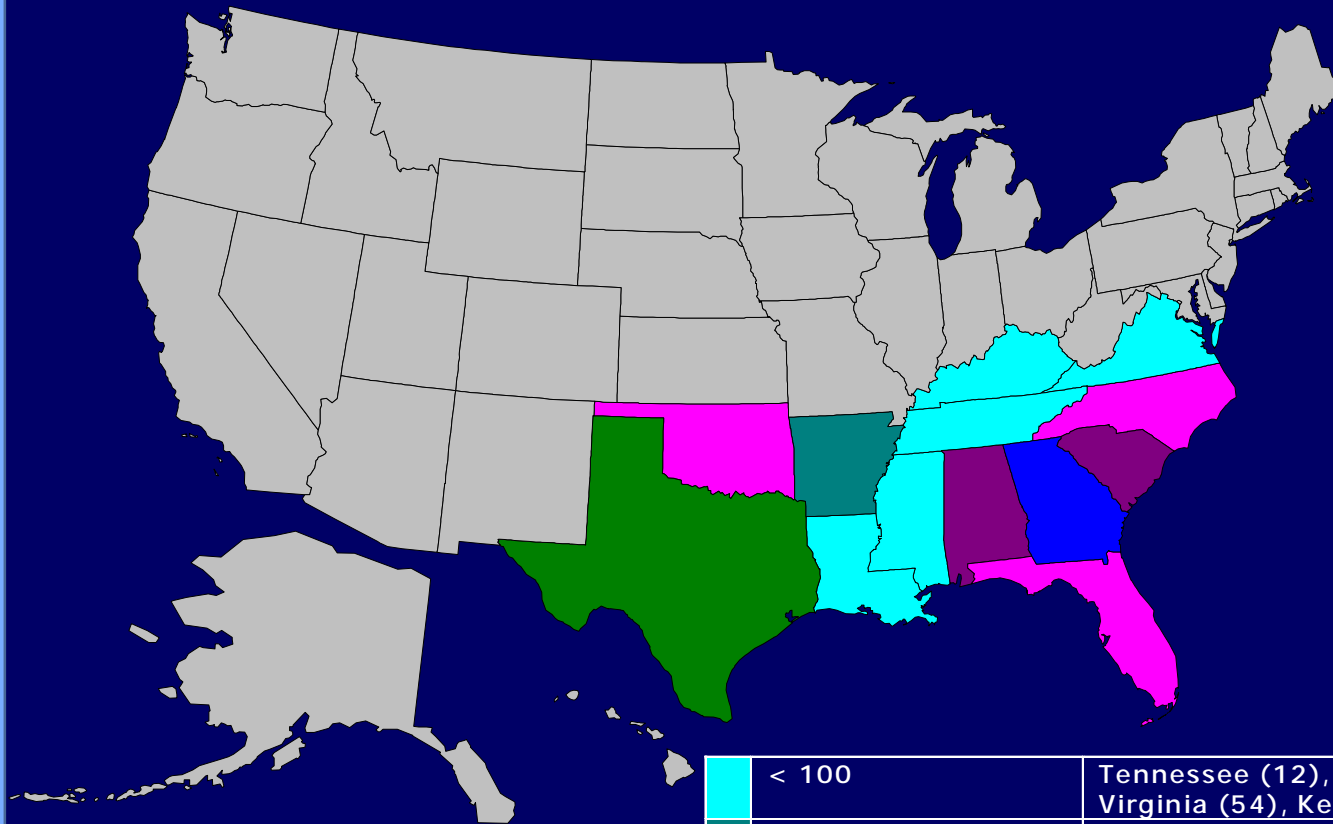
■ Items shipped

– 40,327

■ Orders

– 215

Distributions to S-9 Region in CY2000

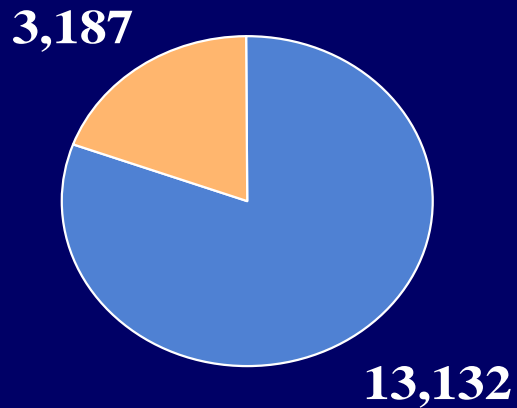


< 100	Tennessee (12), Mississippi (21), Louisiana (39), Virginia (54), Kentucky (83)
>= 100 and < 250	Arkansas (102)
>= 250 and < 500	South Carolina (254), Alabama (333), Puerto Rico (466)
>= 500 and < 1000	Florida (562), Oklahoma (630), North Carolina (849)
> 8500	Georgia (8,592)
> 28000	Texas (28,330)

Average Distributions 1996 – 2000

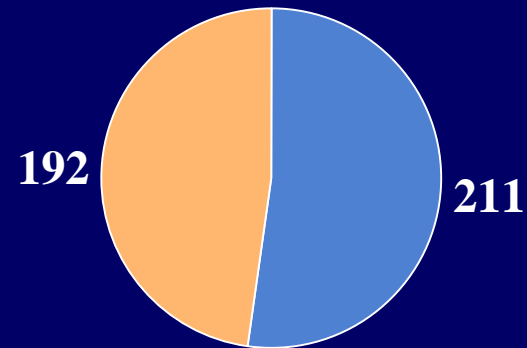
S-9 vs. Domestic

Items Shipped



■ S-9 ■ Domestic

Orders Shipped



■ S-9 ■ Domestic

Foreign Distributions in CY2000

■ Items Shipped

– 5,330

■ Orders

– 118

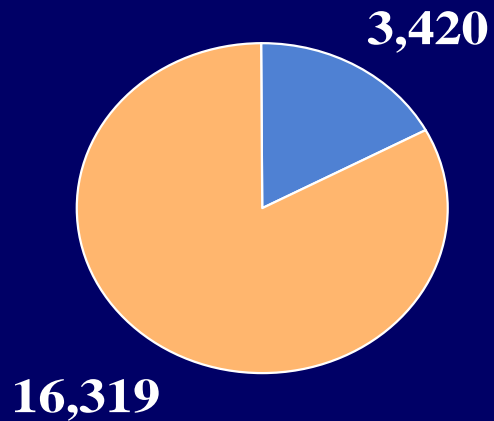
Foreign Distributions in CY2000

Argentina	Czech Republic	Hungary	Kuwait	Poland	Taiwan
Australia	Ecuador	India	Malaysia	Portugal	Thailand
Botswana	Egypt	Indonesia	Moldova	Saudi Arabia	Turkey
Brazil	Estonia	Israel	Netherlands	South Africa	Ukraine
Canada	France	Italy	New Zealand	South Korea	United Arab Emirates
China	Germany	Jamaica	Nigeria	Spain	United Kingdom
Croatia	Honduras	Japan	Philippines	Sweden	Uruguay

Average Distributions 1996 – 2000

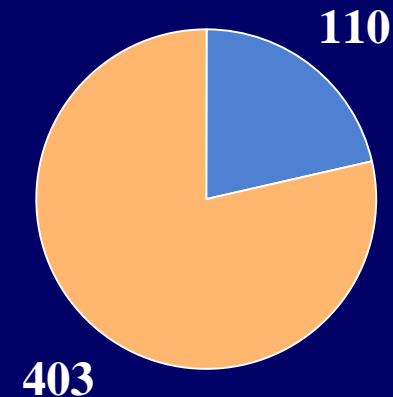
Foreign vs. Domestic

Items Shipped



■ Foreign ■ Domestic

Orders Shipped



■ Foreign ■ Domestic

Future Plans

- Funding
- Staffing
- Unit direction and philosophy

Funding

- ARS increased base funding of PGRCU for FY2001 by \$349,370.
- Normal requirement of adding a scientist with these funds was waived by ARS.
- Germplasm approved by House committee for \$1.5 million increase in FY2002 with Griffin listed as one of the sites.

Staffing

■ Retirements in 2001

- Gil Lovell (Federal agronomist)
- Carolyn Toney (Federal admin. specialist, Athens)
- Rella Harrison (S-9 secretary)

Staffing

- Positions added due to funding increase
 - Biological science technician (currently advertised)
 - » Conduct germination tests.
 - Agricultural research technician (currently advertised)
 - » Support for clover curation and sorghum coordination.
 - Agricultural research technician
 - » Support for grass curation
 - Nine RSA utility workers (hired)
 - » Support for field operations and one worker for each curator
 - Other temporary federal technicians to be added.

Staffing

- Positions replaced due to retirements
 - Agronomist/Botanist (advertised soon)
 - » Cat. 4 scientist to curate warm-season grasses, pearl millet, and bamboo
 - Administrative Technician (currently advertised)
 - » Position based in Griffin instead of Athens
 - S-9 Secretary (advertised soon)

Changes in direction or philosophy

- Curator responsibilities
- Germination testing
- Duplicate identification
- Storage conditions
- Importance of Unit members
- Improvements in procedures and research
- Representation at meetings

Curator responsibilities

■ Pederson

- Curate annual clovers and coordinate sorghum

■ Morris

- Curate special purpose legumes, new crops, and misc. crops (acting curator for warm-season grasses)

■ Agronomist/botanist

- Curate warm-season turf and forage grasses, pearl millet, and bamboo

Germination testing

- Germinations = main priority for new funds.
 - Hire biological science technician.
 - Buy two seed germinators.
 - Redesign space into working seed germination lab.
- Conduct germinations on all new accessions and all seed increases.
- Conduct germinations on all accessions in storage as soon as possible.

Duplicate identification

- PGRCU has 81,660 bags of seed in cold storage but not necessarily 81,660 unique accessions.
- Identify duplicates initially through passport data.
- Link duplicates in GRIN to reduce the total number of accessions.
- Utilize molecular methods to identify genetic diversity and possible duplicate/redundant accessions.

Storage conditions

- Long-term goal is to maximize seed viability under storage.
 - Split samples of all accessions such that the bulk sample is maintained in -18°C storage and a distribution sample is maintained in 5°C storage.
 - Other ideas may also be evaluated.
 - Additional storage facilities may be needed.

Importance of Unit members

- Believe that the mission of the Unit matters.
- Everyone is an integral part of the Unit.
 - State and federal
 - Curators and technicians
 - Farm crew and secretary
 - Seed storage and database manager
- Everyone started off with a clean slate.
- Individual meetings.

Improve procedures and research

- Whole Unit meeting to obtain ideas from all employees on ways to improve Unit procedures.
- Curator meetings to evaluate research program to maximize impact with resources available.
- Continue to explore ideas for cooperation with other researchers.

Representation at meetings

- Crop Germplasm Committee meetings are important and the Unit will be represented.
- Research Leader will attend all nine Crop Germplasm Committee meetings within the first two years.
- Scientists with research appointment will present papers at their national meeting.

Appendix 2

DR. GRAVES GILLASPIE

COWPEA GERMPLASM WITH CMV
RESISTANCE AND HOW THIS RESISTANCE
CAN BE USED TO COMBAT
COWPEA STUNT DISEASE

Cowpea Germplasm with CMV Resistance and How This Resistance Can Be Used to Combat Cowpea Stunt Disease

COWPEA STUNT DISEASE



Results from a co-infection of the plant by BICMV and CMV

COWPEA STUNT DISEASE



Most severe disease of cowpeas in the U.S.

COWPEA STUNT DISEASE



Especially bad in Georgia, Arkansas, and other growing areas of the the southeastern U.S.

POTENTIAL RESISTANT COWPEA FOUND IN 1998 REGENERATION PLOT IN GEORGIA



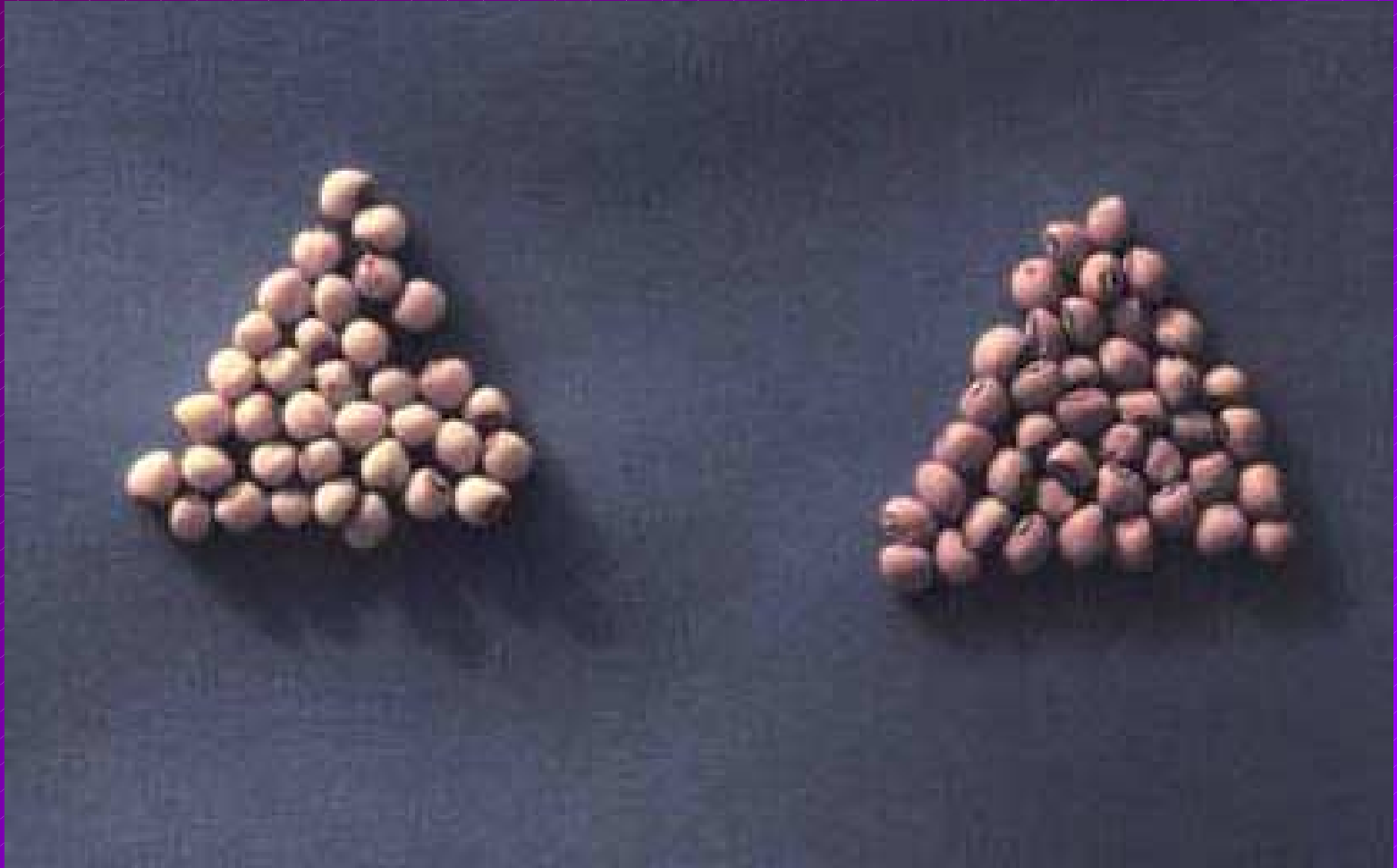
PI 441918 observed to be still green toward end of a growing season.

POTENTIAL RESISTANT COWPEA FOUND IN 1998 REGENERATION PLOT IN GEORGIA



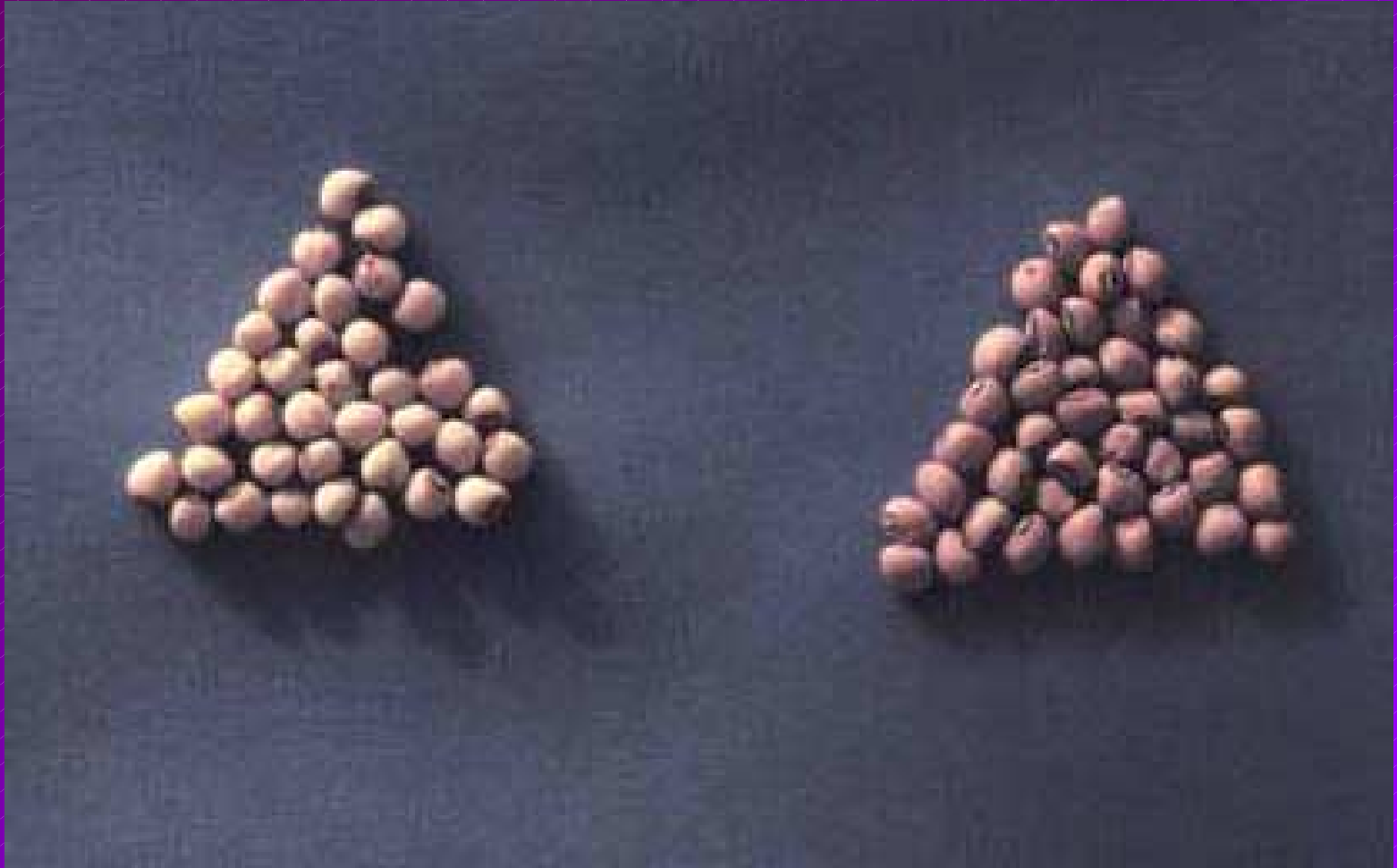
ELISA testing showed that there was no BLCMV
and only one of 20 with CMV.

PI 441918



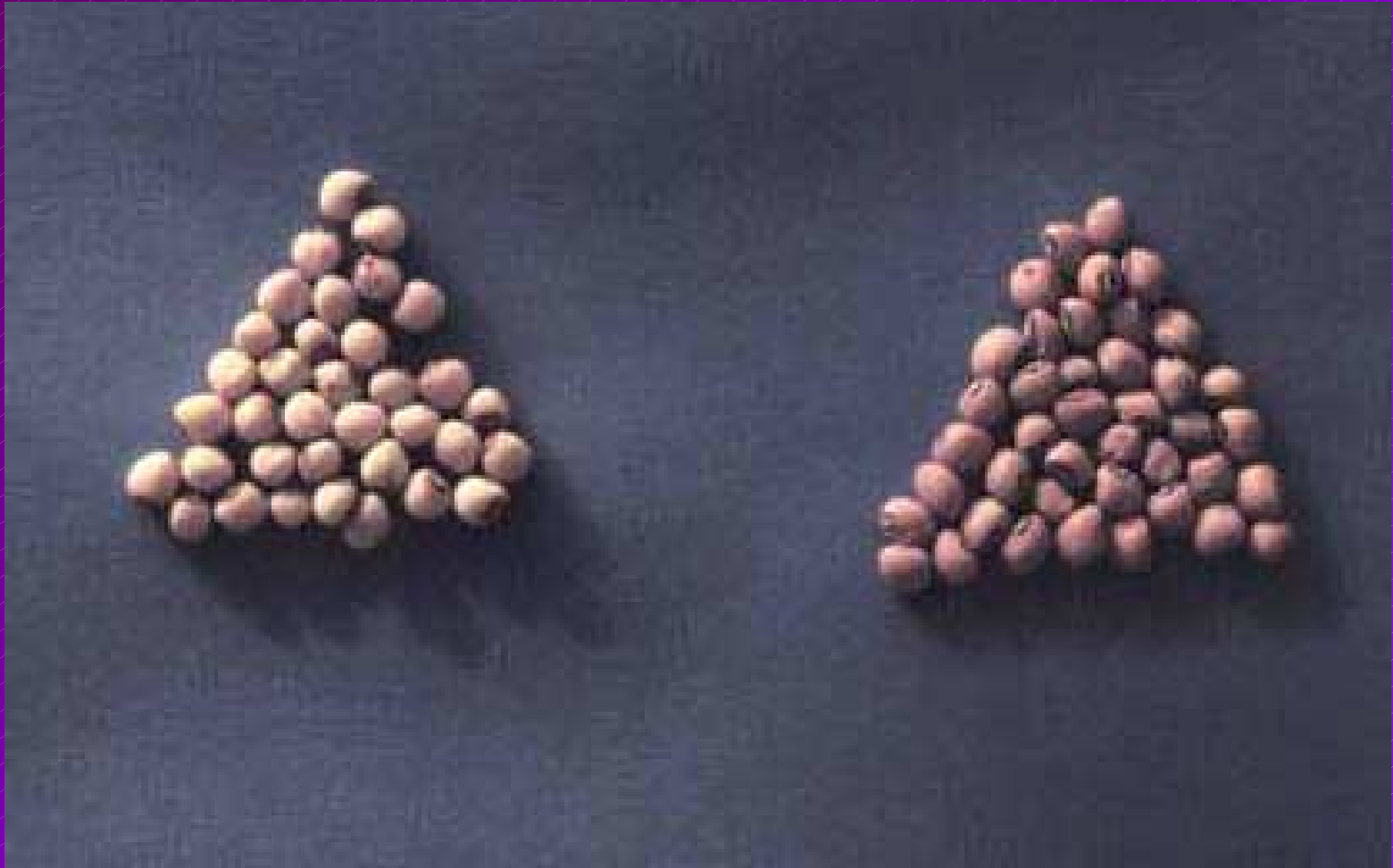
PI 441918 is a mixture of white and tan seeds

PI 441918



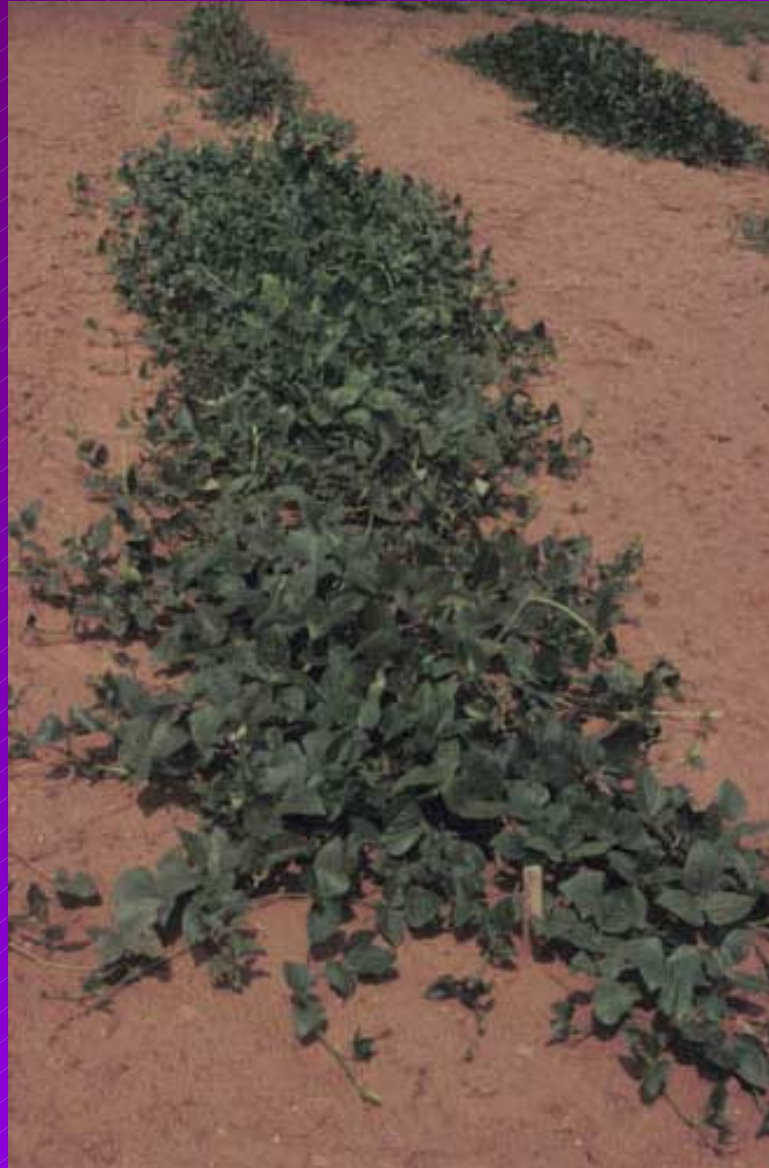
Seeds were separated by color and planted in greenhouse.

PI 441918



The white-seed plants supported a much lower CMV titer than did the control or tan-seed plants. Neither was infected by BICMC.

PI 441918 WHITE AND TAN SEED



PI 441918 TAN SEED



PI 441918 WHITE SEED



Table 1. DAC-ELISA ratings in field tests of cowpea for susceptibility to *Cucumber mosaic virus*.

Cowpea line	Positive plants/ <u>total plants</u>	Mean ^a
Test I, 1 June - 6 August 1999		
PI 441918-white seed	23/45	0.516
PI 441918-tan seed	32/45	0.711
Coronet	44/47	0.935
LSD _{0.01}		0.213
Test II, 5 June - 25 July 2000		
PI 441918-white seed	10/30	0.304
Coronet	22/22	1.000
LSD _{0.01}		0.551
Test III, 17 August - 19 September 2000		
PI 441918-white seed	6/43	0.133
Coronet	69/78	0.870
LSD _{0.01}		0.167

^a Mean across four blocks of positive plants/ randomized block.

CONCLUSIONS

GC-86L-98 (formerly PI 441918 white-seed) was not infected with BICMV in any of tests and had significantly better resistance to infection with CMV than did the susceptible control, Coronet. This large seeded line with a 105-day maturity is a line that has promise for use in breeding to develop a cultivar with cowpea stunt resistance.

Appendix 3

DR. BRAD MORRIS

SPECIAL-PURPOSE LEGUME GENETIC
RESOURCES WITH BIO-FUNCTIONAL USES

Special-Purpose Legume Genetic Resources With Bio-Functional Uses

Brad Morris, Agronomist, Curator

Clarence Lee, Technician

Will Westmoreland, Summer
Assistant

Bio-Functional Legumes

- Guar (*Cyamopsis tetragonoloba*)
- Jackbean (*Canavalia ensiformis*)
- Jicama (*Pachyrhizus erosus*)
- Lablab (*Lablab purpureus*)
- Velvetbean (*Mucuna pruriens*)







C-2631 1 g Protein Lot 000000

SIGMA

CONCAVALIN A

From *Canavalia ensiformis* (Jack Bean)
Type III (1A, 2B, 71-0) EEC No. 215-280

Content: 1 g solid protein
pH 7.0, 10% sucrose
Buffer: 0.1M glycine, 0.1M NaCl
567 g solid
For laboratory use only
Not for human consumption
UNCLASIFIED (GHS) 07-11-12

Product Information

















L49

WB

Unhatched

11-3

H

10-19-00

10-30-00

11-16-00

100 weigh 74.9 gms

L1 1999

Muscuna prot. 2sd.

~~365413~~
365413

TP in 2000-
H 1yr. grown

373
100 seed wt 79.60g

2000

SABINSA CORPORATION
11 Ethel Rd. West WC
BRIDGEWAY, NJ 08804

MUCUNA FRUITED TST 15
LOT#280755Y
NET WT 3.1 KG
FORM-42XC 9-88



Our Innovation Is Your Answer



KOSHER

An aerial photograph of a vast landscape, likely a rural or agricultural area, showing a patchwork of green fields and brown, possibly fallow or harvested, land. The horizon is distant, and the sky is filled with large, white and grey clouds, with a bright light source breaking through near the center, creating a lens flare effect. The overall tone is dramatic and atmospheric.

Additional Bio-Functional Legumes

Kudzu (*Pueraria montana* var.
lobata)

Wingbean (*Psophocarpus*
tetragonolobus)

Estroven[™]

Dietary Supplement
with Natural Estrogens,
Calcium, B6 & B1



The
Natural
way to maintain
a Woman's
Balance

30 Daily Dose Capsules





PHENOLICARPO
FRAGNOLIN
LECTIN I



The Future For Bio-Functional Legumes



Guar

Galactomannan gum

Lowers cholesterol

Reference: Nishimura, 2000,
Bioscience Biotechnology and
Biochemistry, V. 64

Jackbean
Canaline
Antimalarial

Reference: Berger, 2000,
Antimicrobial Agents and
Chemotherapy, V. 44

Lablab
Dolicin
Antifungal and capable of
inhibiting HIV

Reference: Wang and Ng, 2000,
Biochemical and Biophysical
Research Communications, V. 269

Lablab
Dietary fiber
Lowers cholesterol

Reference: Chau and Cheung, 1999,
Nutrition Research, V. 19

Velevetbean
Beta-sitosterol
Lowers cholesterol and prevents
coronary heart disease

Reference: Plat and Mensink, 2001,
Nutrition Metabolism and
Cardiovascular Diseases, V. 11

Velvetbean
Gallic acid
Antimutagenic, anticarcinogenic,
and Anti-inflammatory

Reference: Shahrzad, Aoyagi, Winter,
Koyama, and Bitsch, 2001, Journal of
Nutrition, V. 131

Appendix 4

DR. ROY PITTMAN

IDENTIFICATION OF POLYMORPHIC
MOLECULAR MARKERS IN *Arachis hypogaea*

Identification of Polymorphic Molecular Markers in *Arachis hypogaea*

Melanie Newman and Dr. Roy Pittman

Additional Cooperators - Dr. Tracie Jenkins, Mark Hopkins,
and Dr. Rob Dean

University of Georgia, Plant Genetic Resources Conservation Unit
1109 Experiment Street, Griffin, Georgia 30223

What Are Molecular Markers?

- Markers based on the genetic make-up (DNA) of the plant and thus independent of environmental factors
- When polymorphic they will produce a banding pattern unique to the individual from which the DNA was isolated
- Examples include RFLPs, RAPDs, SSRs, AFLPs, SNPs, etc.

Why Are Molecular Markers Important?

- They allow for marker-assisted breeding.
- They allow a curator to evaluate the genetic diversity within a germplasm collection.
- They allow for the ability to fingerprint new releases.

Current Markers in Cultivated Peanut

- RFLPs and RAPDs are not polymorphic in the cultivated peanut (only in the wild)
- SSRs (limited number - need many more for sufficient polymorphisms to be detected)
- AFLPs have found to be polymorphic

Study Design

- includes the screening of cultivars from each of the six botanical varieties of peanut
- seven SSR markers were used to screen the study population
- data analysis included creating a dissimilarity matrix (Microsat) and a neighbor-joining dendrogram (Phylip) and used AMOVA to estimate within accession variation

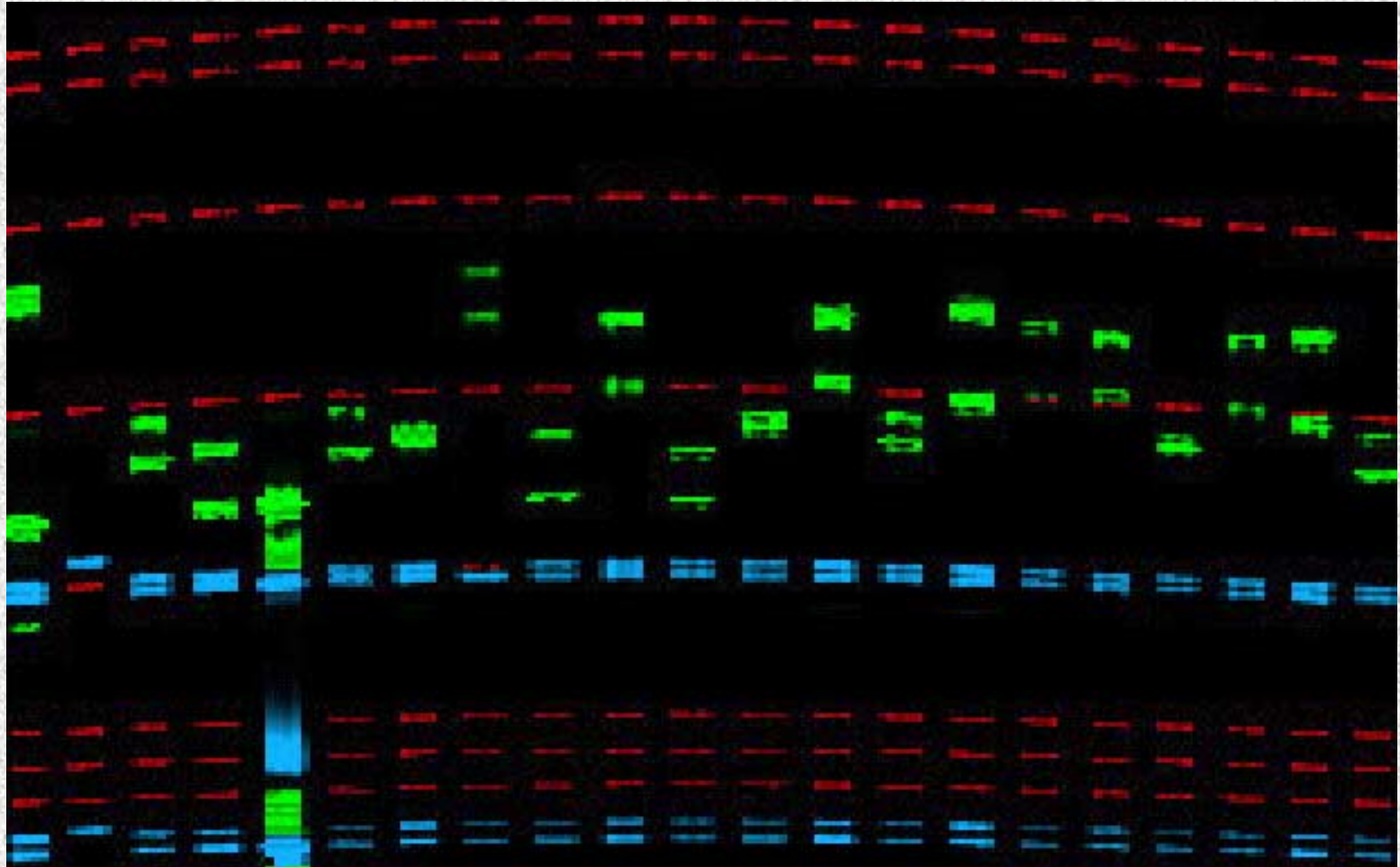
Simple Sequence Repeat


- repetitive sequence of DNA (di, tri, tetra, penta) usually > 6 repeat units
- dispersed throughout the genome
- very polymorphic and thus useful for looking at intraspecific variation
- easy to detect using automated PCR-based protocols

Simple Sequence Repeat

TTACCGATAGGGGGCCCGCCATTATAT
CCGATAGCCTGAGCTTCATATATATA
TATATATATATATATATATATATGC
CCGATTACCTAGGCAGAAATCCGCAT
ATGGCCATCCGGGGCCATACGGGATAT

Peanut SSR gel using primer sets Ah4-026 and Lec-1





North of Mexico City: 4Hi
Mexico City: 2Hi
South of Mexico City: 5Hi

Seclected Peanut SSR Clades

Pichichi, Ecu: 5Hi
Peru: 2Pe

India 1

Peni, Bolivia: 2Pe & 1Fa

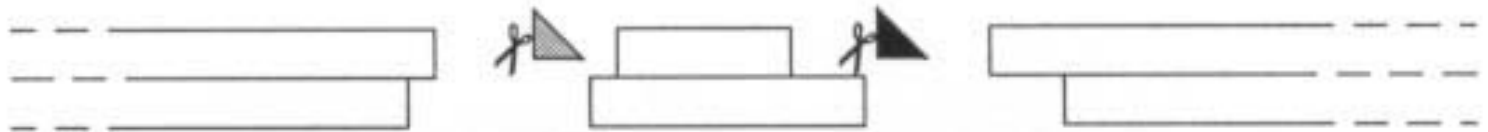
Argentina: Fa & Hy

Preliminary Conclusions

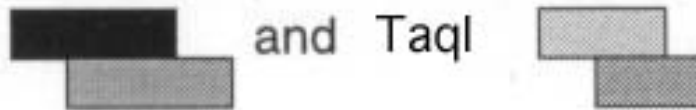
- The current SSR marker system is capable of separating out different cultivars based on geographic location (not by variety)
- Variation does exist within accessions and suggests mixed seeds are present within cultivars in the germplasm system

AFLP Methodology

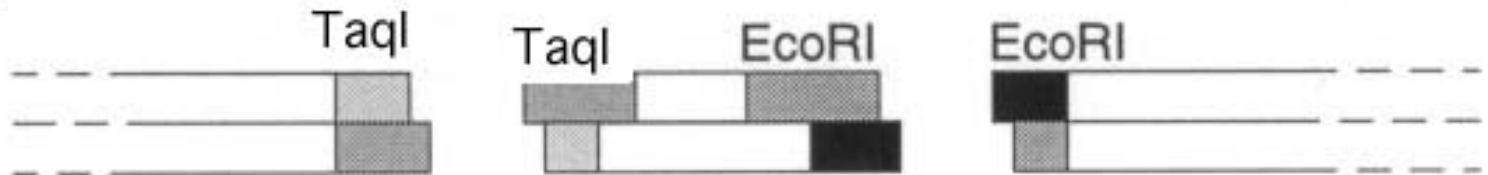
A. Cut genomic DNA into fragments with the restriction enzymes **TaqI** and **EcoRI**:



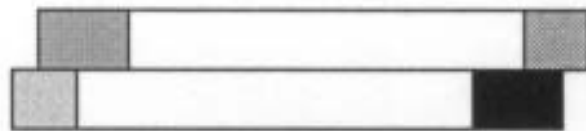
B. Ligate adaptors: **EcoRI** and **TaqI**



C. Modify genomic DNA fragments:



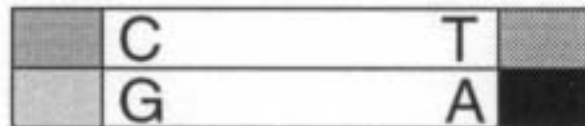
Prepared Template: Genomic DNA
Fragment, Modified with Adaptors




Adaptors,
Core Mix




Thermal
Cycling



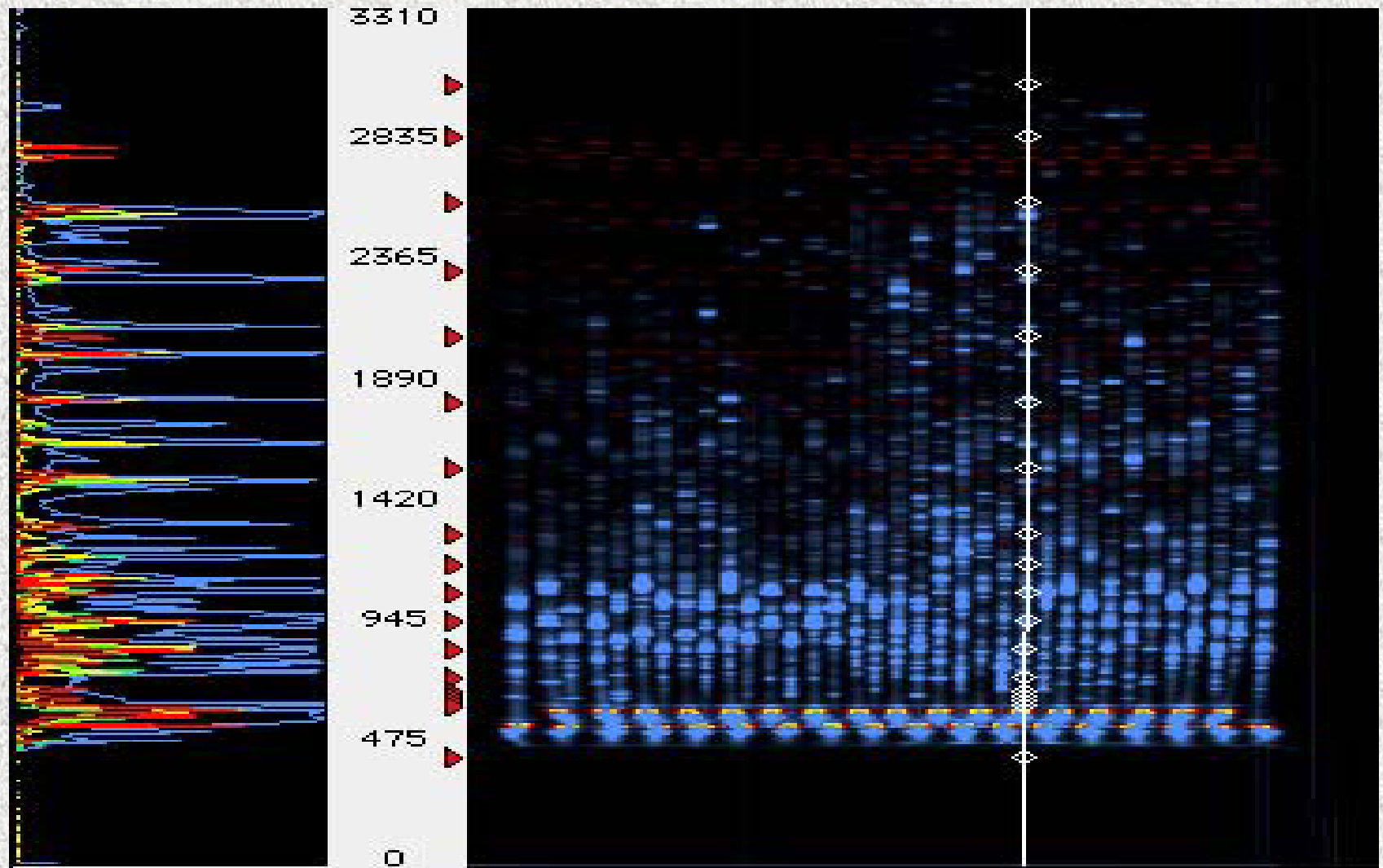
Preselective Primers:

 A : EcoRI adaptor + recognition site + A
or EcoRI adaptor + recognition site

 C : TaqI adaptor + recognition site + C

*Adapted from Applied Biosystems AFLP Manual

AFLP Gel Image on ABI377



Screening Study

A small set of individuals from *Arachis hypogaea* were used in the initial screen which include the following:

- two *hirsutas* (PI 576634 and PI 576613)
- a *fastigiata* (PI 493722)
- a *peruviana* (PI 502088)
- three wild species of *Arachis* (*benthamii*, *batizicoi*, and *villosa*) were screened

The population was screened against 70 AFLP primer sets

AFLPs Evaluated for Polymorphisms

<u>Restriction Enzymes</u>	<u># of Primer Sets Screened</u>	<u>Population Size Screened</u>
EcoRI\Msel	64	Not sure
EcoRI\TaqI	70	4 cult.\3 wild
PstI\Msel	35	40 cult.\3 wild
PstI\TaqI	35	40 cult.\3 wild

Future Plans

- Continue screening to identify more polymorphic markers
- Establish a mapping population for the cultivated peanut
- Enter mapping population into the germplasm system

Appendix 5

DR. BOB JARRET

VEGETABLE CROPS REPORT

Vegetable Crops Report

R. L. Jarret

Curator, Vegetable Crops

Priority

- Defining goals for individual crops (3 to 5 years)

Goals - *Citrullus*

- Continue seed increase at present rate of 150/yr.
 - “Caught up” in about 2 years.
- Then, start on NSSL heirloom cultivars (200)



Goals – *Cucurbita moschata*

- Increase regeneration from 20 to 30 accessions/year.
- Use resources previously allocated to *Abelmoschus*, if required.

Goals - *Capsicum*

- Maintain present regeneration program of 50 accessions/year, or until facilities are improved to handle larger volumes.
- Priority on core accessions and accessions not currently available for distribution.



Goals – Misc. Vine Crops

- Increase regeneration rate to ensure availability of all sp.
- Maintain rate of 10/yr in Griffin
- Move some regeneration to Byron, possibly 10/yr (non-trellised)

Other vegetable crops

- Reduced emphasis on *Abelmoschus* (core only)
- Low emphasis on *S. melongena* and misc. *Solanum* sp.

Goals – *Ipomoea batatas* and related sp.

- Continued in vitro maintenance of all *I. batatas* accessions.
- Possible core collection of *I. batatas* based on AFLPs.
- Core collection could alter maintenance strategy (cyro)
- Increased emphasis on *Ipomoea* sp. seed regeneration in GH during fall-winter months.

Anticipated or Desired Acquisitions

<i>Abelmoschus</i>	- <i>none at this time</i>
<i>Capsicum</i>	- <i>related sp. from Brazil & SA</i>
<i>Citrullus</i>	- <i>related sp. from Namibia and southern Africa</i>
<i>Cucurbita</i>	- <i>none at this time</i>
<i>Ipomoea</i>	- <i>Annual releases from quarantine</i>
Misc. vine crops	- <i>Only to replace lost sp.</i>
<i>Solanum</i>	- <i>Numerous sp. from CA & SA</i>

Present and Future Seed Regeneration Needs

- Improved permanent (?) facilities for seed cleaning, handling, and drying in Byron.
- Equipment to move harvested fruit to cleaning station
- Back-up power for Bldg. 4457 (in vitro *I. batatas*)
- Additional cage covers (X/yr to replace wear)
- Greenhouse benches need to be reset/gravel replaced.